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RHÔNE-POULENC

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September 4, 1992

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Document Processing Center (TS-790)
Attn: Section 8(e) Coordinator (CAP Agreement)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

RE: Report Submitted Pursuant to the TSCA Section 8(e) Compliance Audit Program

CAP ID NO.: 8ECAP - 0004

RP CAP REPORT NO.: RPS - 0198

Dear Sir/Madam:

On behalf of Rhône-Poulenc Inc. (RPI, CN5266, Princeton, NJ 08543-5266) and its subsidiaries, the attached report is being submitted to the Environmental Protection Agency (EPA) pursuant to the Toxic Substances Control Act (TSCA) Section 8(e) Compliance Audit Program (CAP Agreement) executed by RPI and EPA (8ECAP - 0004).

The enclosed report provides information on the following chemical substance:

Chemical Identity: Trimethylolpropane triacrylate (TMPTA)

CAS Registry No: 15625-89-5

CAS Registry Name: 2-Propenoic acid, 2-ethyl-2-[[(1-oxo-2-propenyl) oxy]methyl]-1,3-propanediyl ester

RECEIVED 9/7/95

(2)

The title of the enclosed report is:

> Mutagenicity Evaluation of Trimethylolpropane Triacrylate in the Mouse Lymphoma Forward Mutation Assay

The following is a summary of the adverse effects observed in this report.

> This study is being reported under Section 8(e) CAP because acrylate compounds generally produce positive results in the mouse lymphoma assay. In this study, increases in mutant frequency were observed at concentrations of 1.0 nl/ml and higher without activation and 20.0 nl/ml with activation. Cytotoxicity was seen at all concentrations causing an increase in mutant frequency.

RPI does not claim any portion of the information in this submission to be TSCA confidential business information (TSCA CBI).

RPI has not previously submitted any TSCA Section 8(e) notices or premanufacture notification on the subject chemical substance.

RPI has submitted other studies on this material under the CAP agreement; see RP CAP Report Nos. RPS-0197 and RPS-0270.

On August 15, 1985, Celanese submitted to EPA all available toxicity data on the multifunctional acrylates. However, RPI does not have a detailed list in our records of the reports that were submitted. Therefore, RPI is submitting three copies of the enclosed report and this cover letter: an original and two copies.

Further questions regarding this submission may be directed to Dr. Glenn S. Simon, Director of Toxicology at (919)549-2222 (Rhône-Poulenc, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709).

Sincerely,

Charles E. Moyer Jr

Charles E. Moyer, Jr., Ph.D.
Director, Product Safety
(609)860-3589

CEMjr/mm
Enclosures

LBI ASSAY NO. 3331

MUTAGENICITY EVALUATION OF

TRIMETHYLOLPROPANE TRIACRYLATE

IN THE MOUSE LYMPHOMA FORWARD MUTATION ASSAY

1-12

SUBMITTED TO:

CELANESE CORPORATION
1211 AVENUE OF THE AMERICAS
NEW YORK, NEW YORK 10036

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20989

REPORT DATE: JANUARY, 1979

FINAL REPORT

Litton BIONETICS

PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I-VIII. Items I-IV provide sponsor and compound identification information, type of assay, and the protocol reference number. All protocol references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item V provides the initiation and completion dates for the study, and item VI identifies the tables and/or figures containing the data used by the study director in interpreting the test results. The interpretation itself is in item VII. Item VIII provides the conclusion and evaluation.

The second part of the report, entitled PROTOCOL, describes, in detail, the materials and procedures employed in conducting the assay. This part of the report also contains evaluation criteria used by the study director, and any appendices. The evaluation criteria are included to acquaint the sponsor with the methods used to develop and analyze the test results.

All test and control results presented in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20795.

Copies of raw data will be supplied to the sponsor upon request.

I. SPONSOR: CELANESE CORPORATION

II. MATERIAL (TEST COMPOUND): LBI ASSAY NO. 3331

A. Identification: Trimethylolpropane Triacrylate

B. Date Received: July 6, 1978

C. Physical Description: clear, colorless liquid

III. TYPE OF ASSAY: Mouse Lymphoma Forward Mutation Assay

IV. PROTOCOL NUMBER: 431 (DMT-106)

V. STUDY DATES:

A. Initiation: August 13, 1978

B. Completion: December 19, 1978

VI. RESULTS:

The data are presented in Tables 1 through 3. (pages 5-7). The data show the concentrations of the test compound employed, surviving populations during the expression period, number of mutant clones obtained, and calculated mutation frequencies. All calculations are performed by computer program.

VII. INTERPRETATION OF RESULTS:

The test compound, Trimethylolpropane Triacrylate, was insoluble in water but dissolved in DMSO at a concentration of 500 µl/ml. When this stock was diluted into growth medium, concentrations at 313 nl/ml and higher produced a white precipitate; lower concentrations were soluble. Preliminary cytotoxicity testing without activation indicated variable toxicity and complete lethality at 1.25 to 2.5 nl/ml. Three trials of the mutation assay are reported in which the inclusive applied dose ranges were 0.004875 nl/ml to 5 nl/ml without activation, and 0.004875 nl/ml to 40 nl/ml with activation. The toxicity for each dose was reasonably consistent from trial to trial. For each trial, dose levels showing excessive or insufficient toxicity to cell growth were eliminated from further testing in order to select five doses for completion of the assay that would fall within the range of cytotoxicities (as shown by percent relative growth values after cloning for selection) where any mutagenic activity is normally observed. After the assays were completed, the relative growth in the treated cultures were found to range from 75.2% to 3.6% without activation and

VII. INTERPRETATION OF RESULTS:

from 116.3% to 4.8% with activation among all three trials (Tables 1 to 3).

The results of the mutation assays are presented in Tables 1 through 3.

Without activation, the mutant frequencies in the treated cultures in trial 1 (Table 1) were all comparable to the average of the solvent and untreated negative control values (background frequency), except at the high dose of 1.25 nl/ml. This dose of test compound was very toxic (19.1% relative growth) and the mutant frequency was approximately 3.6 times the background. This response exceeds our criterion of a 2.5-fold minimum increase to establish mutagenic activity at a given dose level. Since mutagenesis was observed only at the highest tested dose, a repeat nonactivation assay was performed for confirmation. In the second trial (Table 2), the same pattern of response was obtained. No increase in mutant frequency occurred at the four lower doses, but at the high dose of 1.0 nl/ml (32.7% relative growth) the mutant frequency was about 4.2 times the background level. Because the background frequency was unusually low in this trial, the assay was repeated again. In the third trial (Table 3) higher doses were tested and more extreme toxicities were obtained. The response observed in this trial was unusual and has no ready explanation. It is clear that mutagenic activity occurred at 1.0 nl/ml and 2.5 nl/ml, but the absence of activity at the intermediate doses suggests that the test compound elicits a variable cellular response and may not always induce mutants. Thus, this trial generally confirms the first two trials in that mutagenic activity is usually observed for doses of 1.0 nl/ml and higher, but the dose-response for these toxic treatments is not defined.

With activation, the first trial (Table 1) was inconclusive. The three lowest doses were only slightly toxic and no increase in mutant frequency was observed. Contamination destroyed the results at the higher doses, but the growth data indicated the applied dose range should be increased to achieve sufficient toxicity. In the second trial (Table 2), no increase in mutant frequency was found for the 1.25 to 10.0 nl/ml dose range, in spite of the high toxicity at 10.0 nl/ml (14.9% relative growth). As noted earlier, however, this trial had unusually low background and positive control mutant frequencies, so the sensitivity of the assay, particularly under activation conditions, may not have been sufficient to detect weak mutagenic activity. A repeat assay (Table 3), with normal mutant frequencies for the controls, showed a 6.4-fold increase in mutant frequency at 20 nl/ml. This dose was highly toxic (4.8% relative growth), whereas little toxicity was obtained with the other tested dose levels. Thus, the results were

VII. INTERPRETATION OF RESULTS:

similar to the nonactivation assay in that mutagenic activity was observed only for very toxic treatments. The toxicity associated with a given dose level appears variable. Also, since about 10-fold higher concentrations of test compound were required to observe toxicity and mutagensis under activation conditions, as opposed to the nonactivation assay, the test compound appears to react with the activation system to form inactive products at the concentrations existing in the assay.

The cloning efficiencies for the solvent and untreated negative controls varied from 61% to 85% without activation and from 67% to 96% with activation among the three trials. Thus, good to excellent cloning conditions were established for the assays.

VIII. CONCLUSIONS:

The test compound, Trimethylolpropane Triacrylate, induced an increase in mutations at the TK locus in L5178Y mouse lymphoma cells in the dose range of 1.0 to 2.5 nl/ml without activation and at 20.0 nl/ml with microsomal activation. This mutagenic response was associated only with doses that were moderately to highly toxic.

Therefore the test compound is considered to be active in the Mouse Lymphoma Forward Mutation Assay.

Submitted by:

Study Director

Brian Myhr 1-24-79

Brian Myhr, Ph.D. Date
Section Chief
Mammalian Genetics
Department of Genetics
and Cell Biology

Reviewed by:

David J. Brusick 1/24/79

David J. Brusick, Ph.D. Date
Director
Department of Genetics
and Cell Biology

Litton BIONETICS

5.__SUMMARY_ [illegible]_LYMPHOMA_(L5178Y)_RESULTS

TABLE 1

A. NAME [illegible] DESIGNATION OF THE TEST COMPOUND: TRIMETHYLOLPROPANE TRIACRYLATE
B. LOT [illegible]: 3331
C. SOLVENT: [illegible]
D. TEST DATE: 10/08/78

TEST	S-9 SOURCE	S-9 TISSUE	DAILY COUNTS 1	DAILY COUNTS 2	DAILY COUNTS 3	RELATIVE SUSPENSION GROWTH (% OF CONTROL)	TOTAL MUTANT CLONES	TOTAL VIABLE CLONES	RELATIVE CLONING EFFICIENCY (% OF CONTROL)	PERCENT RELATIVE GROWTH*	MUTANT FREQUENCY** (X 10E-6)
NONACTIVATION											
SOLVENT CONTROL	---	---	11.6	15.0		100.0	41.0+	256.0	100.0	100.0	16.0
SOLVENT CONTROL	---	---	13.0	14.2		100.0	32.0	237.0	100.0	100.0	13.5
UNTREATED CONTROL	---	---	12.0	16.4		109.8	30.0	177.0	71.8	78.8	16.9
EMS .5 UL/ML	---	---	9.0	8.4		42.2	952.0	82.0	33.3	14.0	1161.0
TEST COMPOUND											
0.078300 UL/ML	---	---	9.0	15.2		76.3	21.0	200.0	81.1	61.9	10.5
0.156000 UL/ML	---	---	13.0	6.6		47.9	28.0	251.0	101.8	48.7	11.2
0.313000 UL/ML	---	---	9.2	12.4		63.6	33.0	187.0	75.9	48.3	17.6
0.625000 UL/ML	---	---	14.0	12.4		96.8	45.0	190.0	77.1	74.6	23.7
1.250000 UL/ML	---	---	6.4	9.4		33.6	77.0	140.0	56.8	19.1	55.0
ACTIVATION											
SOLVENT CONTROL	RAT	LIVER	10.8	17.0		100.0	51.0+	236.0+	100.0	100.0	21.6
SOLVENT CONTROL	RAT	LIVER	11.2	17.2		100.0	75.0	161.0	100.0	100.0	46.6
UNTREATED CONTROL	RAT	LIVER	16.0	15.0		127.6	71.0+	207.0	104.3	133.0	34.3
DMN .3 UL/ML	RAT	LIVER	8.2	9.6		41.8	345.0	47.0	23.7	9.9	734.0
TEST COMPOUND											
0.156 UL/ML	RAT	LIVER	10.8	10.4		105.6	48.0+	191.0	96.2	101.6	25.1
0.313 UL/ML	RAT	LIVER	10.4	15.4		85.1	27.0	194.0	97.7	83.2	13.9
0.625 UL/ML	RAT	LIVER	10.2	14.4		78.1	79.0	236.0	118.9	92.8	33.5
1.250 UL/ML	RAT	LIVER	7.4	20.2		83.8	C	C	----	----	-----
2.500 UL/ML	RAT	LIVER	8.4	12.0		53.6	C	236.0+	118.9	63.7	

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100

** THE RATIO OF CELLS SEEDED FOR MUTANT SELECTION TO CELLS SEEDED FOR CLONING EFFICIENCY IS 10E+4. THEREFORE THE MUTANT FREQUENCY IS: (TOTAL MUTANT CLONES/TOTAL VIABLE CLONES)*10E-4. THE MUTANT FREQUENCY IS GIVEN IN UNITS OF 10E-6.

\+ = ONE PLATE CONTAMINATED, VALUE BASED ON REMAINING TWO PLATES.

C = CONTAMINATED (TWO OR MORE PLATES).

4. SUMMARY OF MOUSE LYMPHOMA ASSAY RESULTS

TABLE 2

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: TRIMETHYLOL PROPANE TRIACRYLATE
B. LBI CODE #: 3331
C. SOLVENT: DIMETHYL SULFOXIDE
D. TEST DATE: 11/12/78

TEST	S-9 SOURCE	S-9 TISSUE	DAILY COUNTS (CELLS/3L X 10E5) 1	2	3	RELATIVE SUSPENSION GROWTH (% OF CONTROL)	TOTAL MUTANT CLONES	TOTAL VIABLE CLONES	RELATIVE CLONING EFFICIENCY (% OF CONTROL)	PERCENT RELATIVE GROWTH*	MUTANT FREQUENCY** (X 10E-6)
NONACTIVATION											
SOLVENT CONTROL	---	---	8.6	7.4		100.0	6.0	190.0	100.0	100.0	3.2
SOLVENT CONTROL	---	---	7.4	13.2		100.0	9.0	282.0	100.0	100.0	3.2
UNTREATED CONTROL	---	---	7.0	8.0		69.4	6.0	290.0	122.9	85.3	2.1
EMS .5 UL/ML	---	---	6.0	5.6		41.7	46.0	252.0	106.8	44.5	18.3
TEST COMPOUND											
0.156 NL/ML	---	---	6.2	7.8		60.0	7.0	209.0	88.6	53.1	3.3
0.313 NL/ML	---	---	9.8	5.2		63.2	5.0	281.0	119.1	75.2	1.8
0.625 NL/ML	---	---	5.6	5.8		40.3	9.0+	250.0	105.9	42.7	3.6
0.750 NL/ML	---	---	5.4	5.4		36.2	7.0	215.0	91.1	32.9	3.3
1.000 NL/ML	---	---	8.8	4.4		48.0	19.0	161.0	68.2	32.7	11.8
ACTIVATION											
SOLVENT CONTROL	RAT	LIVER	8.8	9.8		100.0	3.0	299.0	100.0	100.0	1.0
SOLVENT CONTROL	RAT	LIVER	12.6	8.4		100.0	9.0	346.0	100.0	100.0	2.6
UNTREATED CONTROL	RAT	LIVER	9.0	11.0		103.1	8.0	221.0	68.5	70.6	3.6
DMN .3 UL/ML	RAT	LIVER	4.8	5.2		26.0	75.0+	50.0	15.5	4.0	150.0
TEST COMPOUND											
1.250 NL/ML	RAT	LIVER	12.6	8.6		112.8	3.0+	218.0	67.6	76.3	1.4
2.000 NL/ML	RAT	LIVER	10.6	8.8		97.1	9.0	270.0	83.7	81.3	3.3
2.500 NL/ML	RAT	LIVER	6.6	7.8		53.6	7.0	194.0	60.2	32.2	3.6
5.000 NL/ML	RAT	LIVER	9.4	7.4		72.4	10.0	257.0	79.7	57.7	3.9
10.000 NL/ML	RAT	LIVER	3.2	6.0		18.7	8.0	256.0	79.4	14.9	3.1

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100

** THE RATIO OF CELLS SEEDED FOR MUTANT SELECTION TO CELLS SEEDED FOR CLONING EFFICIENCY IS 10E+4. THEREFORE THE MUTANT FREQUENCY IS: (TOTAL MUTANT CLONES/TOTAL VIABLE CLONES)*10E-4. THE MUTANT FREQUENCY IS GIVEN IN UNITS OF 10E-6.

\+ = ONE PLATE CONTAMINATED, VALUE BASED ON REMAINING TWO PLATES.

4. SUMMARY OF MOUSE LYMPHOMA (L5178Y) RESULTS

TABLE 3

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: TRIMETHYLOLPROPANE TRIACRYLATE
B. LBI CODE #: 3331
C. SOLVENT: DMSO
D. TEST DATE: 12/06/78

TEST	S-9 SOURCE	S-9 TISSUE	DAILY COUNTS (CELLS/ML X 10E5) 1	2	3	RELATIVE SUSPENSION GROWTH (% OF CONTROL)	TOTAL MUTANT CLONES	TOTAL VIABLE CLONES	RELATIVE CLONING EFFICIENCY (% OF CONTROL)	PERCENT RELATIVE GROWTH*	MUTANT FREQUENCY** (X 10E+6)
NONACTIVATION											
SOLVENT CONTROL	---	---	18.6	7.0		100.0	66.0+	170.0	100.0	100.0	38.8
SOLVENT CONTROL	---	---	18.0	8.4		100.0	22.0	156.0	100.0	100.0	14.1
UNTREATED CONTROL	---	---	14.0	10.6		105.5	92.0	227.0	139.3	146.9	40.5
EMS .5 UL/ML	---	---	12.4	5.4		47.6	412.0	67.0	41.1	19.6	614.9
TEST COMPOUND											
1.000 NL/ML	---	---	9.2	6.0		39.2	125.0+	48.0	29.4	11.6	260.4
1.250 NL/ML	---	---	6.4	5.2		23.7	84.0+	236.0+	144.8	34.2	35.6
1.500 NL/ML	---	---	3.8	6.0		12.8	C	102.0+	62.6	8.0	-----
2.000 NL/ML	---	---	4.6	3.6		11.8	74.0+	107.0	65.6	7.7	69.2
2.500 NL/ML	---	---	1.8	9.2		19.6	60.0	30.0	18.4	3.6	200.0
ACTIVATION											
SOLVENT CONTROL	RAT	LIVER	12.4	6.8		100.0	38.0	244.0	100.0	100.0	15.6
SOLVENT CONTROL	RAT	LIVER	12.4	6.6		100.0	20.0+	172.0	100.0	100.0	11.6
UNTREATED CONTROL	RAT	LIVER	9.6	10.6		122.5	33.0	221.0+	106.3	130.1	14.9
DMN .3 UL/ML	RAT	LIVER	9.2	4.6		50.9	108.0+	26.0+	12.5	6.4	415.4
TEST COMPOUND											
2.000 NL/ML	RAT	LIVER	8.8	10.0		105.9	42.0+	183.0	88.0	93.2	23.0
2.500 NL/ML	RAT	LIVER	10.4	9.2		115.2	29.0	210.0+	101.0	116.3	13.8
5.000 NL/ML	RAT	LIVER	6.4	8.8		67.8	44.0	236.0	113.5	76.9	18.6
10.000 NL/ML	RAT	LIVER	10.0	10.0		120.4	47.0+	167.0+	80.3	96.6	28.1
20.000 NL/ML	RAT	LIVER	1.4	3.2		11.6	77.0+	87.0	41.8	4.8	88.5

* (RELATIVE SUSPENSION GROWTH X RELATIVE CLONING EFFICIENCY) / 100
** THE RATIO OF CELLS SEEDED FOR MUTANT SELECTION TO CELLS SEEDED FOR CLONING EFFICIENCY IS 10E+4. THEREFORE THE MUTANT FREQUENCY IS: (TOTAL MUTANT CLONES/TOTAL VIABLE CLONES)*10E-4. THE MUTANT FREQUENCY IS GIVEN IN UNITS OF 10E-6.

+ = ONE PLATE CONTAMINATED, VALUE BASED ON REMAINING TWO PLATES.

C = CONTAMINATED (TWO OR MORE PLATES).

PROTOCOL NO. 431

1. OBJECTIVE

The objective of this study is to evaluate the test material for its ability to induce forward mutation in the L5178Y TK+/- mouse lymphoma cell line, as assessed by colony growth in the presence of 5-bromo-2'-deoxyuridine (BrdU).

2. RATIONALE

Thymidine kinase (TK) is a cellular enzyme that allows cells to salvage thymidine from the surrounding medium for use in DNA synthesis. If a thymidine analog such as BrdU is included in the growth medium, the analog will be phosphorylated via the TK pathway and be incorporated into DNA, eventually resulting in cellular death. Cells which are heterozygous at the TK locus (TK+/-) may undergo a single step forward mutation to the TK -/- genotype in which little or no TK activity remains. Such mutants are as viable as the heterozygotes in normal medium because DNA synthesis proceeds by de novo synthetic pathways that do not involve thymidine as an intermediate. The basis for selection of the TK-/- mutants is the lack of any ability to utilize toxic analogs of thymidine, which enables only the TK-/- mutants to grow in the presence of BrdU. Cells which grow to form colonies in the presence of BrdU are therefore assumed to have mutated, either spontaneously or by the action of a test substance, to the TK-/- genotype.

3. MATERIALS

A. Indicator Cells

The mouse lymphoma cell line, L5178Y TK+/-, used in this assay is derived from the Fischer L5178Y line of Dr. Donald Clive. Stocks are maintained in liquid nitrogen and laboratory cultures are periodically checked for the absence of mycoplasma contamination by culturing methods. To reduce the negative control frequency (spontaneous frequency) of TK-/- mutants to as low level as possible, cell cultures are exposed to conditions which select against the TK-/- phenotype (exposure to methotrexate) and are then returned to normal growth medium for three or more days before use.

B. Media

The cells are maintained in Fischer's mouse leukemia medium supplemented with L-glutamine, sodium pyruvate, and horse serum (10% by volume). Cloning medium consists of the preceding growth medium with the addition of agar to a final concentration of 0.35% to achieve a semisolid state. Selection medium is cloning medium containing 50 or 100 µg/ml of BrdU.

3. MATERIALS (continued)

C. Control Compounds

1. Negative Controls

A negative control consisting of assay procedures performed on untreated cells is performed in all cases. If the test compound is not soluble in growth medium, an organic solvent (normally DMSO) is used; the final concentration of solvent in the growth medium will be 1% or less. Cells exposed to solvent in the medium are also assayed as the solvent negative control to determine any effects on survival or mutation caused by the solvent alone. For test substances assayed with activation, the untreated and solvent negative controls will include the activation mixture.

2. Positive Controls

Ethylmethane sulfonate (EMS) is highly mutagenic via alkylation of cellular DNA and will be used at 0.5 µl/ml as a positive control for nonactivation studies.

Dimethylnitrosamine (DMN) requires metabolic activation by microsomal enzymes to become mutagenic and will be used at 0.3 µl/ml as a positive control for assays performed with activation.

D. Sample Forms

Solid materials are dissolved in growth medium, if possible, or in DMSO, unless another solvent is requested. Liquids are tested by direct addition to the test system at predetermined concentrations or following dilution in a suitable solvent.

4. EXPERIMENTAL DESIGN

A. Dosage Selection (Cytotoxicity testing)

The solubility of the test chemical in growth medium and/or DMSO is first determined. Then a wide range of chemical concentrations is tested for cytotoxicity, starting with a maximum applied dose of 10 mg/ml for test chemicals soluble in media or 1 mg/ml for solutions in organic solvents. After an exposure time of four hours, the cells are washed and a viable cell count is obtained the next day. Relative cytotoxicities expressed as the reduction in growth compared to the growth of untreated cells are used to select seven to ten doses that cover the range from 0 to 50-90% reduction in 24-hour growth. These selected doses are subsequently applied to cell cultures prepared for mutagenicity testing, but only four or five of the doses will be carried through the mutant selection process. This procedure compensates for daily variations in cellular cytotoxicity and ensures the choice of four or five doses spaced from 0 to 50-90% reduction in cell growth.

B. Mutagenicity Testing

1. Nonactivation Assay

The procedure used is based on that reported by Clive and Spector (1975) and is summarized as follows. Cultures exposed to the test chemical for four hours at the preselected doses are washed and placed in growth medium for two or three days to allow recovery, growth and expression of the induced TK-/- phenotype. Cell counts are determined daily and appropriate dilutions are made to allow optimal growth rates.

At the end of the expression period, 3×10^6 cells for each selected dose are seeded in soft agar plates with selection medium and resistant (mutant) colonies are counted after 10 days incubation. To determine the actual number of cells capable of forming colonies, a portion of the cell suspension is also cloned in normal medium (nonselective). The ratio of resistant colonies to total viable cell number is the mutant frequency.

A detailed flow diagram for the mutation assay is provided in Figure 1.

2. Activation Assay

The activation assay can be run concurrently with the nonactivation assay. The only difference is the addition of the S9 fraction of rat liver homogenate and necessary cofactors (CORE) during the four-hour treatment period. CORE consists of NADP (sodium salt) and isocitric acid. The final concentrations of the activation system components in the cell suspension are: 2.4 mg NADP/ml; 4.5 mg isocitric acid/ml; and 50 µl S9/ml.

C. Preparation of 9,000 x g Supernatant (S9)

Fischer 344 male rats are normally used as the source of hepatic microsomes. Induction with Aroclor 1254 or other agents is performed by injections five days prior to sacrifice. After decapitation and bleeding, the liver is immediately dissected from the animal using aseptic technique and placed in ice cold 0.25M sucrose buffered with Tris at pH 7.4. When an adequate number of livers is obtained, the collection is washed twice with fresh buffered sucrose and completely homogenized. The homogenate is centrifuged for 10 minutes at 9,000 x g in a refrigerated centrifuge and the supernatant (S9) from this centrifuged sample is retained and frozen at -80°C until used in the activation system. The S9 fraction may be obtained from induced or noninduced rats or other species, as requested.

EVALUATION CRITERIA

A compound is considered mutagenic in this assay if:

- A dose-response relationship is observed over 3 of the 5 dose levels employed.
- The minimum increase at the low level of the dose-response curve is at least 2.5 times greater than the solvent and/or negative control values.
- The solvent and negative control data are within the normal range of the spontaneous background for the TK locus.

All evaluations of mutagenic activity are based on consideration of the concurrent solvent and negative control values run with the experiment in question. Positive control values are not used as reference points, but are included to ensure that the current cell population responds to direct and promutagens under the appropriate treatment conditions.

Occasionally, a single point within a concentration range will show an increase 2.5 times greater than the spontaneous background. If the increase is at the high dose, is reproducible, and if an additional higher dose level is not feasible because of toxicity, the chemical can be considered mutagenic. If the increase is internal within the dose range and is not reproducible, the increase will normally be considered aberrant. If the internal increase is reproducible, several doses clustered around the positive concentration will be examined to either confirm or reject the reliability of the effect.

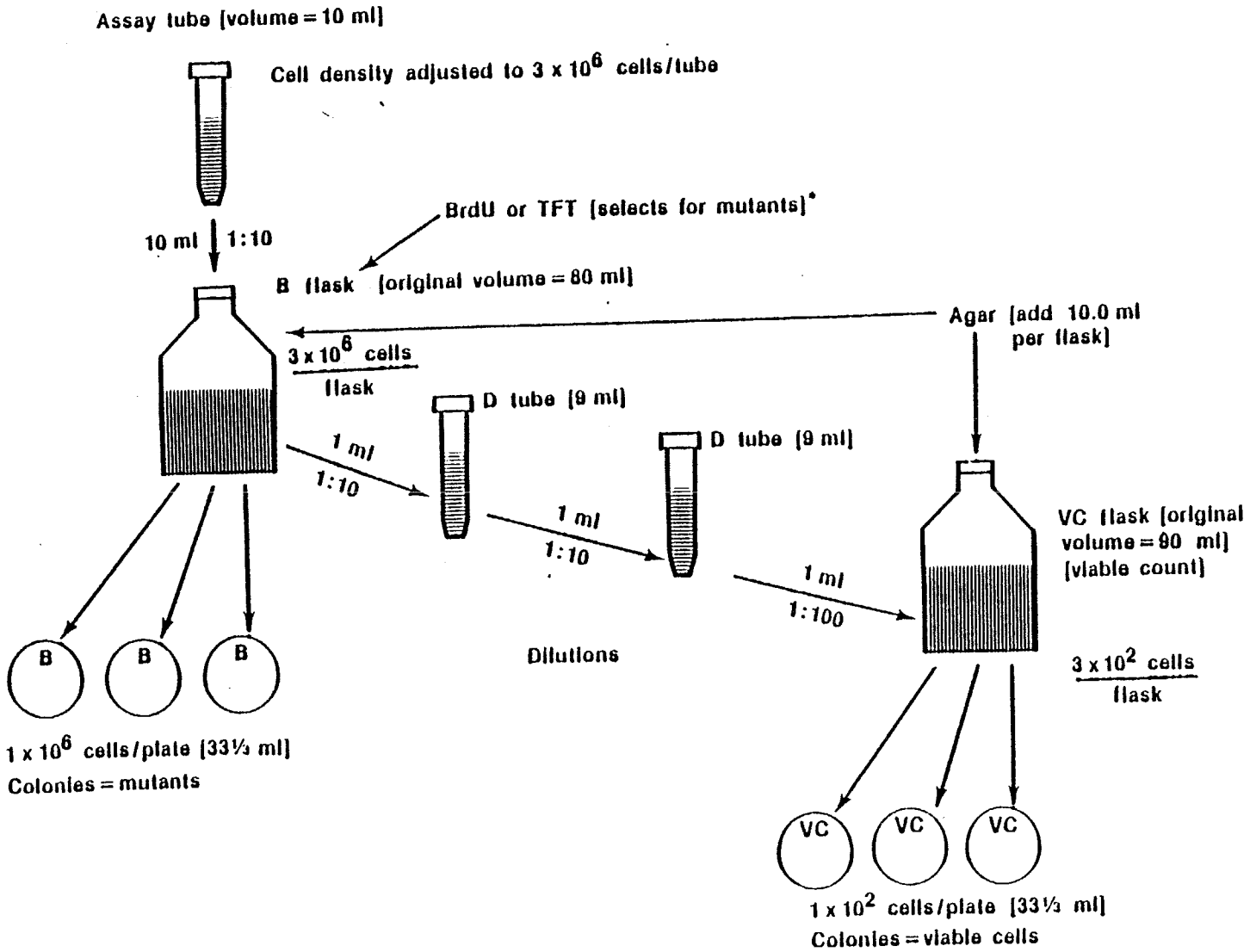
As the data base on the assay increases, the evaluation criteria can be expected to become more firmly established.

5. REPORT

The screened doses, cell counts, and mutant and viable colony counts will be entered into a computer program. The results are analyzed and printed.

6. REFERENCE

Clive, D. and Spector, J.F.S.: Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mutation Res., 31:17-29, 1975.



*Added after removal of 1 ml for viable count dilutions.

FIGURE 1. LYMPHOMA CLONING FLOW CHART

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Charles E. Moyer, Jr., Ph.D.
Director, Product Safety
Rhône-Poulenc Inc.
CN 7500
Cranberry, New Jersey 08512-7500

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

APR 0 6 1995

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information and **refer to the reverse side of this page for "EPA Information Requests"** .

All TSCA 8(e) submissions are placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA §8(e) policy statement (43 FR 11110, March 16, 1978). Confidential submissions received pursuant to the TSCA §8(e) Compliance Audit Program (CAP) should already contain information supporting confidentiality claims. This information is required and should be submitted if not done so previously. To substantiate claims, submit responses to the questions in the enclosure "Support Information for Confidentiality Claims". This same enclosure is used to support confidentiality claims for non-CAP submissions.

Please address any further correspondence with the Agency related to this TSCA 8(e) submission to:

Document Processing Center (7407)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan

Terry R. O'Bryan
Risk Analysis Branch

Enclosure 120884

Triage of 8(e) Submissions

Date sent to triage: 12/8/95

Submission number: 12088A

NON-CAP (CAP)

TSCA Inventory: (Y) N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX (GTOX)

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

For Contractor Use Only

entire document: 0 1 2 **pages** 1,2 **pages** 1,2, labs

Notes:

Contractor reviewer : LPS **Date:** 3/10/95

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 0992 -12088 SEQ. A

TYPE: (INT) SUPP FLWP

SUBMITTER NAME: Rhone - Poulenc Inc.

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)
DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
(0678) CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/USERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 09/04/92 OTS DATE: 09/22/92 CSRAD DATE: 02/07/95

CHEMICAL NAME:

CAS#
15625-89-5

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
(0204)	MUTA (IN VITRO)	01 (02) 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
0224	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
(0243)	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA:

NON-CBI INVENTORY
(YES)
NO
DETERMINI

ONGOING REVIEW
YES (DROP/REFER)
NO (CONTINUE)
REFER

SPECIES
In Vitro

TOXICOLOGICAL CONCERN:
LOW
(MED)
HIGH

USE:

PRODUCTION:

CAS SR

COMMENTS

21) 8EHQ-92-12088: Rank - medium.

Chemical: trimethylolpropane triacrylate {2-ethyl-2-[[(1-oxo-2-propenyl)oxy]methyl]-1,3-propanediyl ester of 2-propenoic acid; TMPTA: CAS# 15625-89-5}.

Mutagenicity evaluation of trimethylolpropane triacrylate in the mouse lymphoma forward mutation assay, Litton Bionetics, Inc., Kensington MD, dated January 1979: Positive for gene mutations in L5178Y $TK^{+/-}$ mouse lymphoma gene mutation assay <u>in vitro</u> both with and without metabolic activation.